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# Detecting sub-clinical transplant rejection using minimally-invasive miRNA and mRNA signature

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To date over 650,000 solid-organ transplantations have been conducted in the United States. While there have been considerable advances in graft clinical management, and immunosuppression therapies (ISTs) over the last two decades, the rates of acute rejection are still significant. Highly invasive needle biopsies of transplanted allografts are stull the current gold-standard for pathological assessment of rejection. These are expensive and painful procedures, with inter-observer variability in biopsy readings being a common issue, and irreversible damage of upwards of 50% of patient's kidney function can occur by the time clinical symptoms manifest, which impacts the rates of time-to-graft-loss as well as patient survival.

Non-invasive miRNA and mRNA signatures are rapidly advancing towards the clinical setting as they are demonstrating the ability to detect subclinical rejection weeks to months before clinical manifestation – and thus offer huge potential to increase graft survival. This presentation will outline our recent multicenter prospective NIH-sponsored Clinical Trials in Transplantation (CTOT) 3, 4 and 7 studies, which show early detection of rejection in liver and kidney transplant settings many weeks before clinical manifestation.

A number of urinary biomarker studies to date have shown strong promise to diagnose and even prognosticate acute rejection in the kidney allograft setting (PMID: 23968332; 23822777 and 28121909). Recent advances in omic technologies, and aggregation of large appropriate clinical samples from transplant studies such as iGeneTRAiN (www.igenetrain.org and PMID: 26479416), aims to accelerate development of such biomarker studies to remove the need for highly-invasive and painful biopsies, and more importantly identify subclinical rejection before irreversible graft damage occurs.

The advantage of using whole urinary omics is that any measured phenotype such as: metabolite products from patients gut microbiota; prescription drugs; and decline in kidney function such as the estimated Glomerular Filtration Rate (eGFR), can be assessed at a deep molecular level in a prospective manner.The large international transplant consortium iGeneTRAiN's aims to improve transplantation success through the discovery of immunological and genetic markers underpinning rejection and other complications post-transplant. After successfully generating the largest genome-wide genetic datasets ever assembled in transplant genomics, iGeneTRAiN has laid the groundwork for performing similarly large multi-'omic' analyses on different solid organ transplant studies. We present here a number of previous and ongoing studies that show the utility of minimally invasive biomarkers in the kidney transplant setting.

Over 14,000 liver transplants are performed worldwide yearly. The ability to sub clinically identify liver graft recipients on ACR trajectories vs those with successful withdraw from IST using minimally invasive, robust, biomarkers with high specificity and sensitivity would a major advance in personalized patient care. We performed miRNA profiling of 752 transcripts on 318 serum

samples from 90 liver recipients from the ITN-030 & CTOT-03 NIH prospective studies. 48 recipients randomized to IST-withdrawal were analyzed for prediction of rejection, and to identify those who could tolerate lower IST.

Serum miRNA profiles at time-of-biopsy from 104 samples with and without biopsy proven ACR were compared in a two-stage study. 15 miRNAs were observed to be significantly associated with ACR diagnosis after multiple testing corrections (FDR-adjusted $p<0.05$). A logistic regression model consisting of 3 miRNAs was identified for differentiation of ACR from non-ACR (AUC=0.90, 95%CI=0.84-0.95, 92.6% sensitivity & 84.2% specificity, $p=0.0001$). We tested this signature in independent validation samples(sera from 19 ACR & 16 non-ACR patients), replicating ACR vs non-ACR differentiation (AUC of 0.89 [95%CI: 0.83 - 0.94], 84% sensitivity & 75% specificity, $p=0.01$). Statistically significant alterations in this 3 miRNA ACR panel preceded the rejection event by up to 40 days. The composite score of another distinct 3 miRNA panel early after initiation of IST minimization (at 75% pre-withdrawal dose) identified those with significant IST reduction (<25% of pre-withdrawal dose, AUC=0.88 [95%CI: 0.80 - 0.95], sensitivity=0.82, specificity=0.90, $p=0.02$). We demonstrate that two distinct multi-marker miRNAs signatures from sera can be used to: diagnose ACR up to 40 days before the manifestation of clinical symptoms; predict rejection trajectories; and guide IST minimization.